Reference: *Biol. Bull.*, **160:** 280–291. (April, 1981)

THE INFLUENCE OF TEMPERATURE ON THE KINETICS OF ALLOGRAFT REACTIONS IN A TROPICAL SPONGE AND A REEF CORAL*

I. S. JOHNSTON,[1] P. L. JOKIEL,[2] C. H. BIGGER,[1] AND W. H. HILDEMANN[1]

[1]*Immunogenetics Group, School of Medicine and Dental Research Institute, University of California, Los Angeles, California, 90024, USA; and*
[2]*Hawaii Institute of Marine Biology, Kaneohe, Hawaii 96744, USA*

Abstract

Many tropical sponges and reef-building corals demonstrate highly discriminating transplantation immunity when grafted with allogeneic tissue. The speed of rejection changes seasonally, and therefore the role of temperature was investigated. Replicate parabiotic allografts of a Hawaiian sponge, *Callyspongia diffusa*, and a reef-coral, *Montipora verrucosa*, were exposed to three different temperature regimens: approximately 23°, 25°, and 27°C. The primary allograft reactions were extremely temperature dependent in both species; the median reaction times (MRT) for the first-set grafts at summer temperatures (27°C) were approximately half the winter temperature (23°C) MRTs. Significantly accelerated second-set reactions in the coral were similarly affected by temperature; however, maximally accelerated second-set reactions in the sponge were similar at all three temperatures. The primary mean reaction times for replicate grafts varied significantly among the individual genotypic combinations brought into parabiosis. Furthermore, different genotypic combinations responded to temperature changes with qualitatively distinctive reaction trends as well as different reaction times. Parabiotic allografts mimic intraspecific interactions that are a consequence of natural contact through growth of sedentary marine animals. The influence of temperature on the outcome of intraspecific competition suggests that temperature fluctuations could maintain high levels of genetic polymorphism within individual populations of sponges and corals.

Introduction

The phenomena of self versus not-self recognition and of immunocompetence among marine invertebrates are of broad interdisciplinary interest. They are relevant to considerations of: (1) ecological competition, *e.g.* intraspecific and interspecific competition for space among sedentary animals (Connell, 1976; Jackson and Buss, 1975; Bigger, 1980); (2) population genetics, *e.g.* the benefits and maintenance of genetic polymorphism and strategies for sexual versus asexual reproduction (Shick and Lamb, 1977); (3) pathogenesis and defense against pathogenic organisms; (4) symbiosis, *e.g.* the specificity and stability of algal-invertebrate symbioses (Trench, 1979), and (5) immunology, *e.g.* phylogenetic trends in immune systems (Manning and Turner, 1976).

Intimate and prolonged tissue contact between conspecific but genotypically

Received 17 October 1980; accepted 23 December 1980.
Abbreviations: MRT, median reaction time; COCO, KYC, collection sites.
* Contribution No. 594 of the Hawaii Institute of Marine Biology.

nonidentical individuals, *i.e.* allogeneic individuals, in certain reef corals (Raison *et al.*, 1976; Hildemann, Raison, Cheung, *et al.*, 1977) and sponges (Hildemann *et al.*, 1979; Van de Vyver, 1980) can generate immune-type transplantation rejection. Allogeneic graft rejection results in zones of tissue death occurring unilaterally or more commonly bilaterally at the contact interface. These sponge and coral reactions are characterized by discriminating allogeneic recognition and subsequent cytotoxicity. At least some species display accelerated responses towards subsequent contact with the same allogeneic individuals, in a way that indicates immunological memory (Hildemann *et al.*, 1979; Hildemann, Bigger, Jokiel, *et al.* 1980; Hildemann, Jokiel, *et al.* 1980; Evans *et al.*, 1980). We believe that these properties of sponge and coral allograft reactions fulfill minimal criteria for their full identification with other immunological phenomena (Hildemann, Bigger, Jokiel, *et al.*, 1980).

In higher animals, allo-transplantation reactions are usually induced artificially by surgical means, though maternal-fetal incompatibilities occur naturally in many mammals. Such allogeneic incompatibilities probably express surveillance mechanisms that normally detect and destroy antigenically transformed, potentially cancerous cells, or pathogenic microorganisms. Among sedentary benthic marine animals, allogeneic tissue contacts or grafts are a natural result of vegetative growth, especially in encrusting organisms. When contact occurs during coral and sponge growth, alloreactive soft tissues do not fuse and a vacant zone of variable width is created between the colonies (Hildemann, Raison, and Hull, 1977; Curtis, 1979). This outward expression may at first sight appear equivalent to the end results of certain xenogeneic, or interspecific, interactions among animals competing for the same space or feeding upon one another. However, the underlying mechanisms that achieve and maintain separation may be quite different (see Lang, 1973; Hildemann, Raison, Cheung, *et al.*, 1977).

In ectothermic invertebrates it is not surprising to find physiological responses that depend quantitatively and even qualitatively on temperature. Much work has been done with corals to determine the effect of temperature on growth and mortality (Jokiel and Coles, 1977; Houck *et al.*, 1977), calcification (Clausen and Roth, 1975), photosynthesis and respiration (Coles and Jokiel, 1977), and reproduction (Jokiel and Guinther, 1978). Synergistic effects of temperature with light and salinity have also been investigated (Coles and Jokiel, 1978). However, little is known about general temperature effects in sponges (Sarà and Vacelet, 1973) other than an effect on pumping rates (Reiswig, 1971). Nothing was known about how temperature changes might affect allograft reactions in sponges and corals until we observed that they rejected allografts more rapidly at higher ambient sea water temperatures during the summer (Hildemann *et al.*, 1979). Other workers have now also become alert to the possibility that ambient temperature changes may significantly affect alloincompatibilities in sponges (Evans *et al.*, 1980; Van de Vyver, 1980). The close dependence of diverse immune responses on environmental temperature has long been known in ectothermic fishes, amphibians, and reptiles (Hildemann, 1962). The published accounts of sponge grafting experiments carried out in temperate vs. tropical areas need explanation, because some reports from cooler areas have not noted alloincompatibility and/or concomitant cytotoxicity (Moscona, 1968; Paris, 1961). Here, we report the substantial effect of temperature on alloreactivity under controlled laboratory conditions in the tropical sponge *Callyspongia diffusa* and the reef-coral *Montipora verrucosa* over the normal Hawaiian range of 22–28°C.

Materials and Methods

We conducted our experiments at the Hawaii Institute of Marine Biology (Coconut Island, Oahu) in fiberglass aquaria ($1 \times 1 \times 0.5$ m) in full natural sunlight shaded to 30% of incident illumination with black nylon window screen. The aquaria were constantly aerated and supplied with non-recirculating unfiltered sea water pumped from Kaneohe Bay at a rate sufficient to flush them in less than 1 h. Each of three identical arrays of aquaria were subject to different temperature regimens: approximately 23°C, 25°C, and 27°C. The 25°C experiment was simply exposure to the ambient temperature of the incoming water. In the 23°C experiment, the incoming water was chilled by 2°C below ambient while in the 27°C experiment, the incoming water was heated by 2°C. Only inert materials contacted the sea water; pump and piping surfaces were of non-toxic plastic; heat exchange surfaces were of titanium and glass. Temperatures were measured daily with a mercury thermometer calibrated to 0.1°C. The aquarium temperatures followed the slight (less than 1°C) natural diurnal temperature cycle observable on the reefs, with afternoon temperatures slightly warmer than those observed in the morning. Table I shows ambient temperatures, and the precision with which the cooler and warmer treatments were controlled. The range of sea water temperatures over which these experiments were performed is well within the yearly range of monthly means of 22–28°C for Kaneohe Bay (*cf.* Jokiel and Coles, 1977, Fig. 3). A seasonal warming trend in ambient temperatures was only marginally evident during the experments.

The corals and sponges used in the experiments were collected from two widely separated reefs in Kaneohe Bay, to prevent comparisons of genetically similar or identical animals derived vegetatively from the same parent colony. One site was a shallow patch reef north of Coconut Island (henceforth referred to as COCO). The second reef was located across the Bay, some 3 km to the south near the Kaneohe Yacht Club (henceforth referred to as KYC). The two sites are separated by a deep (10 m) lagoon with a sand or mud bottom unsuitable for the species studied. Any cross colonization must therefore be through dispersal of motile larval forms.

Table I

Ambient temperatures and temperature control during the course of Callyspongia diiffusa *and* Montipora verrucosa *grafting experiments.*

	Mean ambient temperature (°C ± SD) 25°C Treatment		Mean of temperature differences from ambient (°C ± SD) (simultaneous measurements made at any time of day between 07:15 and 17:30 h)	
	Morning 07:30–10:30 h	Afternoon 13:00–16:00 h	23°C chilled treatment	27°C heated treatment
Data collected over the duration of the sponge experiment: 19 April–9 May 1979	25.1 ± 0.3	25.8 ± 0.3	−2.3 ± 0.5	+2.1 ± 0.2
Data collected over the duration of the coral experiment: 23 April–8 July 1979	25.3 ± 0.4	25.9 ± 0.5	−2.3 ± 0.6	+1.9 ± 0.4

The two species studied occur on reefs at both sites. *Callyspongia diffusa* is a large, violet, branched sponge found on shallow subtidal reef flats and not in deeper water. The reef coral *Montipora verrucosa* occurs in such shallow areas, but is more abundant along the deeper portions of the reef face. This species is one of the most polymorphic species of coral known, assuming encrusting, branching, massive, or foliacious (plate-like) growth forms (Vaughan, 1907).

Eight large colonies of *C. diffusa* were obtained from each site. The COCO colonies were numbered C1–C8 and the colonies from KYC C13–C20. Six branch tips, approximately 6–8 cm long, were excised from each parent colony. Branch tips from different colonies were grafted together by immobilizing them in contact with each other, as previously described by Hildemann *et al.* (1979). Each piece of sponge from colony C1 was grafted with a piece from colony C13, and so on with the combinations C2–C14 through C8–C20. The grafts interfaced by at least 2–3 cm of linear contact between the presumed allogeneic sponge pairs. The grafts did not involve cut surfaces of sponge tissue, but rather intimate physical contact of intact pinacoderm surfaces, mimicing natural contact grafts. We have called these "parabiosis grafts". Each replicated set of graph pairs ($n = 6$) is equivalent to replicated grafts between two different, highly inbred populations of laboratory animals. The six replicates of each interclonal combination were equally divided among each of the three temperature regimens.

Large colonies of the flat-plate growth form of *M. verrucosa* were also harvested from each site. Using heavy bone shears, we cut each colony into six genetically identical plates, each with 6–10 cm of intact growing edge from the original colony. Parabiosis grafts were set up with at least 3–4 cm of intimate skeletal and soft tissue contact interface along undamaged growing edges (after Hildemann, Raison, Cheung, *et al.*, 1977). The numbering of clones, total number of allografts, and assignment of pairs to the three temperature regimens was the same as described for *C. diffusa*, with the prefix M rather than C.

We observed the daily progress of alloreactions in both sponge and coral grafts until grafts were definitively rejected, and the time to this point was recorded as the rejection time (days). We chose an objectively convenient end point, i.e. when the soft tissues were killed back to 1 mm on either or both sides of a graft interface. In both species, allogeneic killing reveals naked underlying skeletal materials: a brown spongin fiber network in *C. diffusa* (Fig. 1; *cf.* Hildemann *et al.*, 1979, Fig. 1B), and the white calcium-carbonate corallum in *M. verrucosa* (Fig. 2; *cf.* Hildemann, Raison, Cheung, *et al.*, 1977, Fig. 2). Using suitable magnification aids, we measured the extent of tissue killing with an accuracy of 0.1 mm. Following definitive reactions in all "first-set" grafts in a given temperature treatment, these graft pairs were separated and immediately regrafted together at new interfaces, i.e. as "second-set" grafts. The progress of each second-set graft was similarly recorded until definitive rejection.

Since second-set graftings could only be made when the primary reactions were complete, second sets could not be started simultaneously, as were the first-set grafts. Therefore, second-set replicates in each temperature treatment were not necessarily concurrent. This was not a major concern because the temperature regimens were stable.

Results

Tables II and III give first- and second-set mean reaction times for the replicated grafts in each temperature treatment. The differences among reaction times for

FIGURE 1. Allogeneic killing in an incompatible parabiotic graft of the sponge, *Callyspongia diffusa*. A bilateral cytotoxic reaction has exposed the spongin fiber skeletal framework at the contact interface (between apposing arrow heads).

identical interclonal grafts at the same temperature were small, as previously shown for *C. diffusa* (Johnston and Hildemann, in press). In the present experiment, 7 of 24 replicated pairs of first-set *M. verrucosa* grafts had identical reaction times while 10 more had reaction times within 1–4 days of each other. On the other hand, reaction times for grafts assembled from different interclonal combinations differed considerably: for example, compare mean reaction times between the sponge first-set combinations C3–C15 and C4–C16, and between the coral first-set combinations M4–M16 and M8–M20. Therefore, at a given temperature the cytotoxic reactivity provoked by allogeneic cell-surface contact among random pairs of sponges or corals was reflected in a continuum of reaction times: some were faster and some were slower depending on the genotypic combination of the individual graft pairs. Certain pairings consistently reacted rapidly at all temperatures while others reacted more slowly.

Since the same combinations were replicated at each temperature, we made paired comparisons to look for an overall temperature effect in spite of the variation introduced by different genotypic graft sources. Table IV summarizes the results of paired-comparisons *t* tests performed on the reaction-time differences for each of the two temperature increments in both first- and second-set grafts, and also on the first-set to second-set differences within each temperature treatment. For both sponges and corals, first-set reaction times decreased significantly over each temperature increment, *i.e.* between 23°C and 25°C, and again between 25°C and 27°C. By contrast, over the same temperature increments neither species showed

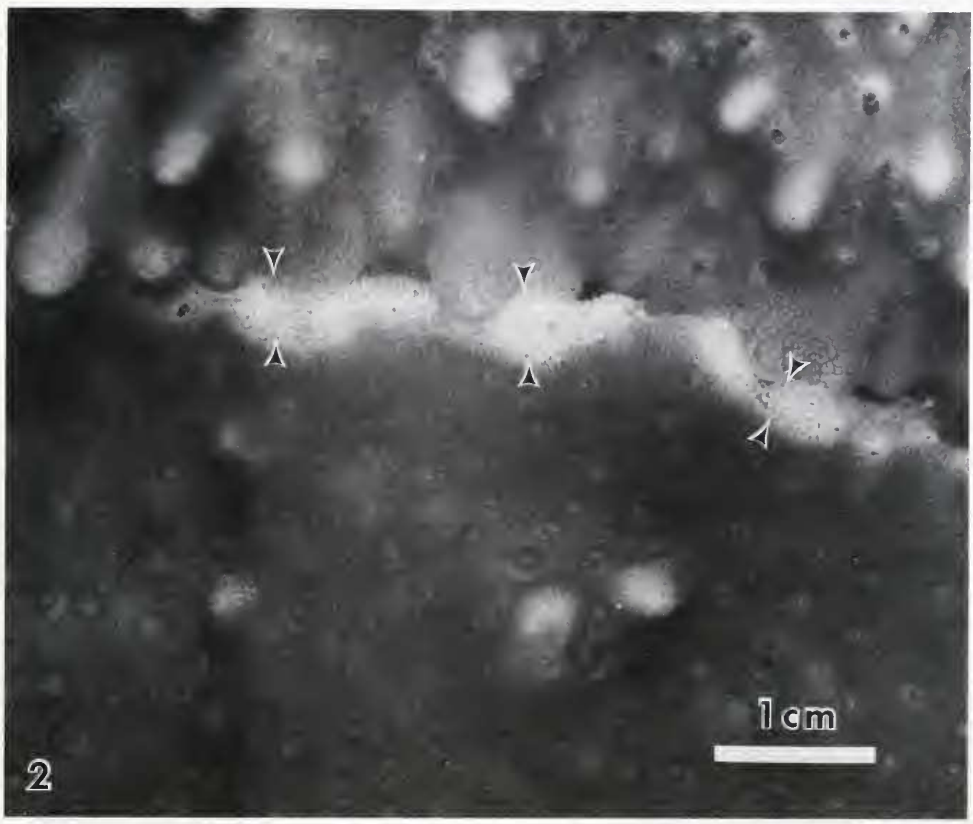

FIGURE 2. Allogeneic killing in an incompatible parabiotic graft of the reef-coral, *Montipora verrucosa*. In the lower individual a unilateral cytotoxic reaction at the contact interface has exposed the underlying calcium carbonate corallum (between apposing arrow heads).

a significant temperature effect on second-set reaction times. In both animals at a given temperature, second-set reactions were significantly faster than first-set reactions, except for coral allografts at the warmest (27°C) temperature. Two-way analysis of variance for first-set reaction times in both animals showed significant variation resulting from (i) temperature, (ii) individual graft pairing, *i.e.* genotypic combination, and (iii) interaction between temperature effect and genotypic combination. This latter interaction is illustrated by a comparison between the trends in first-set mean reaction times over the three temperatures in coral combinations M1–M13 and M6–M18.

Graphic plots of median reaction times (derived from log-normal transformations of the data from the eight different genetic combinations tested for each animal) versus experimental temperature means (Fig. 3) illustrate the general trends in the effect of temperature on allograft reactions. Except for the sponges' strikingly accelerated second-set reactions, which were similar at all three temperatures, the grafts generally were rejected faster with increasing temperature. In other words, the sharply heightened immune reactions against repeat sponge grafts were much less affected by temperature than were the reactions involved in the primary response. In the more slowly reacting coral allografts, however, both

TABLE II

Reaction time for replicate interclone allografts of the sponge Callyspongia diffusa.

	Mean Reaction Time (days) n = 2					
	1st Set			2nd Set[a]		
Clone Combination	23°C	25°C	27°C	23°C	25°C	27°C
C1–C13	9.0	8.0	4.5	4.0	3.5	3.5
C2–C14	14.0	10.0	8.0	4.0	6.0	5.0
C3–C15	15.5	13.5	8.0	2.5	6.0	4.0
C4–C16	8.0	5.5	5.0	3.0	N.A.[c]	4.0
C5–C17	13.0	8.5	8.0	4.0	2.5	4.5
C6–C18	10.0	11.0	10.0	3.5	5.0	5.0
C7–C19	15.0	11.0	11.0	4.0	2.5	4.5
C8–C20	13.5	11.0	8.5	2.0	3.0	4.0
Median Reaction Time (days) for the 8 clone combinations	13.0	10.0	8.0	3.5	3.5	4.0
Estimate of parametric median from log–normal transformation of data[b] (days)	11.5	8.9	6.7	3.7	3.6	3.8

[a] Regrafting was accomplished after 11 days for the 27°C experiment and after 16 days in both the 25°C and 23°C experiments.

[b] From the method of Litchfield (1949).

[c] N.A.: not available since sponge C4 was completely killed by the first-set alloreaction in both replicates.

first- and second-set rejection times were temperature dependent. Extrapolating the curvilinear relationship of *M. verrucosa* first-set reaction times indicates that at temperatures below 21°C, cytotoxic allograft rejection might not occur in this coral population.

Our method of scoring definitive graft rejection obscures the fact that allogeneic killing may occur in one or both partners. The sponge combination C4–C16, for example, produced conspicuous unilateral reactions in all grafts; C4 was always killed back by over 1 mm before any sign of cytotoxic damage appeared on C16, if any appeared at all. Bilateral killing was much more common, however, indicating reciprocal interactions at the graft interfaces.

DISCUSSION

Allograft reactivity is the expression of mechanisms that normally (a) preserve the genetic integrity of the adult individual, *e.g.* preventing chimera formation that would otherwise happen if allogeneic tissues fused compatibly, and (b) promote the success of an individual genotype, sometimes at the expense of allogeneic neighbors, *e.g.* in intraspecific competition for space. Over the temperature range investigated in these experiments, the rate of this type of intraspecific immune interaction, in both coral and sponge, is temperature dependent; at higher temperatures primary transplantation rejection occurs faster. However, note the unexpectedly prolonged reaction times at 25°C, compared to 23°C, in the M1–M13, M4–M16 and M5–M17 combinations of *M. verrucosa.* Because different genotypes react quite differently to the same temperature changes, a qualitative aspect of intraspecific com-

TABLE III

Reaction time for replicate interclone allografts of the coral Montipora verrucosa.

	Mean Reaction Time (days) n = 2					
	1st Set			2nd Set[a]		
Clone Combination	23°C	25°C	27°C	23°C	25°C	27°C
M1–M13	16.0	19.0	13.0	15.0	9.0	4.0
M2–M14	43.0	18.0	12.0	23.0	17.0	24.0
M3–M15	21.0	12.0	10.0	16.0	9.0	4.0
M4–M16	11.0	15.0	9.0	5.0	4.0	3.0
M5–M17	17.0	18.5	10.0	9.0	9.0	6.0
M6–M18	31.0	8.0	8.0	6.0	3.0	4.0
M7–M19	29.0	12.0	14.5	11.5	12.0	4.0
M8–M20	51.5	28.0	24.0	15.0	17.0	24.0
Median Reaction Time (days) for the 8 clone combinations	21.0	15.0	10.0	12.0	9.0	4.0
Estimate of parametric median from log-normal transformation of data (days)	23.3	15.0	10.6	10.5	7.0	4.3

[a] Regrafting in the 27°C experiment was completed 16–29 days after first set grafting. The corals in the 25°C experiment were regrafted 22–29 days after the time of the first set grafting while the 23°C regraftings were made 34–55 days after the first set.

petition or adaptive immunity is also temperature dependent. Therefore, by giving a competitive or survival advantage to certain individuals, ambient sea water temperature may well affect the physical and genetic structures of coral and sponge populations.

We believe that at a given temperature the degree of allo-incompatibility, as measured by the time it takes to achieve an arbitrarily defined reaction end point, depends on the polymorphism of certain crucial gene loci. In equivalent well-studied systems in mammals, histoincompatibility depends not only on the number of mis-

TABLE IV

Results of paired-comparison t *tests for first and second-set mean reaction times for replicate allografts of* Callyspongia diffusa *and* Montipora verrucosa.

	Level of Significance (*P* value)	
Comparison	*C. diffusa*	*M. verrucosa*
23°C 1st set to 25°C 1st set	0.01*	0.04*
25°C 1st set to 27°C 1st set	0.02*	0.02*
23°C 2nd set to 25°C 2nd set	0.50	0.07
25°C 2nd set to 27°C 2nd set	>0.50	0.67
23°C 1st set to 23°C 2nd set	0.01*	0.01*
25°C 1st set to 25°C 2nd set	0.01*	0.01*
27°C 1st set to 27°C 2nd set	0.01*	0.06

* Significant at 95% confidence level.

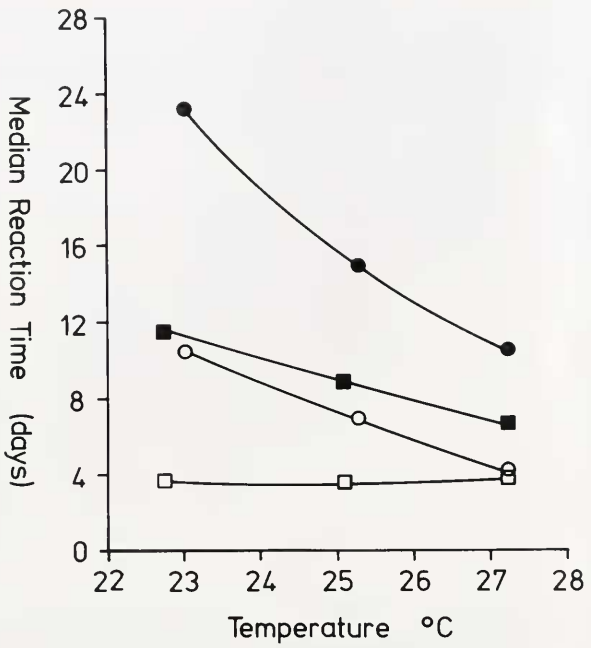

FIGURE 3. Relationship between mean morning temperature and estimated parametric median reaction time for *Montipora verrucosa* first-set grafts (solid circles), *M. verrucosa* second-set grafts (open circles), *Callyspongia diffusa* first-set grafts (solid squares) and *C. diffusa* second-set grafts (open squares).

matches at these loci but also on the individual properties of each mismatched gene; some genes, or rather their gene products, may provoke a more vigorous cytotoxic reaction either by an allogeneic graft partner, or directed at an allogeneic graft partner (Snell *et al.*, 1976). Our previous experiments with corals and sponges (Hildemann, Bigger, Johnston, *et al.*, 1980; Hildemann, Bigger, Jokiel, *et al.*, 1980; Hildemann, Johnston, *et al.*, 1980) imply that both are highly polymorphic at multiple histocompatibility loci, and also that they can sometimes be characterized either as "killers" or as particularly susceptible individuals.

Although certain genotypes may have an intraspecific competitive advantage at a given temperature, environmental temperatures fluctuate in the short and long terms. It is assumed that a high degree of genetic polymorphism within a population insures the preservation of the species despite extreme temperature perturbations (*cf.* Weins, 1977; Shick and Lamb, 1977).

Various attempts have been made to explain how diversity is maintained in coral reef environments (*e.g.*, Jackson and Buss, 1975; Buss, 1976; Connell, 1976). Although most of the discussion has been concerned with interspecific diversity, many of the concepts are transferable to intraspecific diversity (genetic polymorphism). In the absence of some intervening factors, a simple ranked hierarchy of allograft reactivity, such that individual A out-competes individual B, and B out-competes C (A→B→C), would obviously lead to eventual elimination of the lower ranked individuals. Jackson and Buss (1975) propose the possiblity of "competitive networks" in terms of different forms of competition (*i.e.* toxicity and overgrowth) such that A→B→C→A. In his work with corals on the Great Barrier Reef, Connell (1976) did not find the circular network patterns essential to the competitive net-

work theory. His data best fit a "transitive network, external disturbances" model. In that system, A→B→C, but the higher ranked species is more susceptible to outside disturbances (*e.g.*, hurricanes). Therefore, at intervals, the number of higher-ranked individuals would decrease, allowing the lower ranked to increase, and thereby stabilize the system. However, another mechanism for maintaining diversity is suggested by earlier data and by this present study. Connell (1976; interspecific, corals), Buss (1976; interspecific, sponges, corals, bryozoans, coralline algae, foraminifera), and Bigger (1980; intraspecific, sea anemones), while showing the predictable outcome of some interactions, have noted a number of variable outcomes, i.e. A⇆B. In Connell's (1976) study, a predictable A→B only occurred in a minority of cases. The present study demonstrated a genotypically variable histoincompatible response at a given temperature and shows that such responses vary as the temperature changes. Since the experimental temperatures were well within the normal annual fluctuations of this area, annual temperature cycles could influence and shift incompatibility rankings such that A→B, A⇆B, A↹B, A←B, or some variation, would occur sequentially. This could explain Connell's (1976) "standoffs" (inter- and intraspecific). Longer-term temperature cycles also might account for his reports of coral A overgrowing B one year, then B over A the second year, A over B the third year, etc. This kind of mechanism, which shifts competitive rankings as a consequence of normal cyclic ecological factors, could function in conjunction with major disturbances or, in some systems, could possibly maintain diversity without major external disturbances. These hypotheses of competition/ecological factor interactions require definitive field studies to substantiate their relevance to natural populations.

As a result of its niche on shallow reef tops, *C. diffusa* experiences greater temperature extremes than *M. verrucosa*. However, the more eurythermal sponge is only slighlty less sensitive than the more stenothermal coral with respect to the overall effect of temperature on primary alloreactions, i.e. both animals approximately doubled their first-set median reaction times between 27°C and 23°C. By comparison, coral growth can change 10% per 1°C (Jokiel and Coles, 1977), whereas planulation in the coral *Pocillopora damicornis* diminishes by up to an order to magnitude in response to a 1°C change from a 26–27°C optimum (Jokiel and Guinther, 1978). From field studies with three Caribbean sponges, Reiswig (1971) recorded reductions of 13–35% in overall pumping rates with an approximately 2°C decrease in ambient temperature.

Insofar as alloreactivity may be just one apsect of immunocompetence in these animals, then temperature sensitivity may also characterize other immune-type reactions. The ability to combat pathogenic organisms and the recognition of and control over populations of potential symbionts and commensals may well be reduced as temperature decreases. Low temperature immunosuppression for *M. verrucosa* from Kaneohe Bay, at about 21°C, would be significant for this animal since the low-temperature lethal limit is about 18°C (Jokiel and Coles, 1977). Therefore, temperature sensitivity of immune-type reactions could be one factor determining the restricted distribution of tropical sponges and corals.

We believe that allograft reactions are the end products of at least two basic underlying processes: allorecognition and induced cytotoxicity. Temperature may affect these processes in quite different ways, and therefore overall reaction rates may reflect complex multivariate relationships. In sponges, allograft reactions typically proceed through an intitial stage of tissue fusion (Paris, 1961; Evans *et al.*, 1980). In *C. diffusa*, we have termed this stage "tissue bridging" (Hildemann, Bigger, Johnston *et al.*, 1980) to distinguish it from long-term compatible tissue

fusion; after only 4–6 hours in contact, allograft fusion is morphologically distinct from the fusion of compatible isografts. This stage probably accounts for reports of compatible allograft fusion (*i.e.* a failure of allorecognition: Moscona, 1968; McClay, 1974) especially since it may persist longer at lower temperature. Tissue bridging in *C. diffusa* rarely lasts more than 5 days at 25°C, whereas in *Hymeniacidon perleve* at 5–15°C it may persist for more than 14 days, although it is abolished at 2°C (Evans *et al.*, 1980).

The prominence of induced cytotoxicity in sponge allografts seems especially dependent on ambient temperature. Paris (1961) did not see cytotoxic reactions in orthotopic allografts of two species of Mediterranean sponge when seasonal water temperatures were lowest (11°C). However, at other times of the year, when ambient temperatures were above 16°C, the grafts and their hosts rapidly became necrotic. Van de Vyver (1980) also notes that for parabiotic allografts of *Axinella polyploides*, cytotoxic reactions are rapidly and clearly apparent to the naked eye at 23°C, but at 20°C cytotoxicity is only detectable by histological analysis, giving an initial false impression that the grafts are compatibly fused. In the present experiments, coral and sponge replicate graft pairs developed conspicuously larger areas of allogeneic killing in the 27°C treatment than in the 23°C treatment. *In situ* allograft reactions are generally more prominent in the tropics than in temperate areas.

In the future, temperature manipulation could be used to "dissect" immunological function in these animals much the same as in ectothermic vertebrates (e.g. Wright *et al.*, 1978). The different temperature sensitivity of first-set versus second-set alloreactions implies different sequences of events. The species differences in second-set reaction kinetics between corals and sponges could also imply different underlying mechanisms and controls.

Acknowledgment

This research was supported by NIH Grants CA 23025 and AI 15705.

LITERATURE CITED

Bigger, C. H. 1980. Interspecific and intraspecific acrorhagial aggressive behavior among sea anemones, a recognition of self and not-self. *Biol. Bull.* **159:** 117–134.

Buss, L. W. 1976. Better living through chemistry: the relationship between allelochemical interactions and competitive networks. Pp. 315–327 in F. W. Harrison and R. R. Cowden, Eds., *Aspects of sponge biology*. Academic Press, New York.

Clausen, C. D., and A. A. Roth. 1975. Effect of temperature and temperature adaptation on calcification rate in the hermatypic coral *Pocillopora damicornis*. *Mar. Biol.* **33:** 93–100.

Coles, S. L., and P. L. Jokiel. 1977. Effects of temperature on photosynthesis and respiration in hermatypic corals. *Mar. Biol.* **43:** 209–216.

Coles, S. L., and P. L. Jokiel. 1978. Synergistic effects of temperature, salinity and light on the hematypic coral *Montipora verrucosa*. *Mar. Biol.* **49:** 187–195.

Connell, J. H. 1976. Competitive interactions and the species diversity of corals. Pp. 51–58 in G. O. Mackie, Ed., *Coelenterate ecology and behavior*. Plenum Press, New York.

Curtis, A. S. G. 1979. Individuality and graft rejection in sponges, a cellular basis for individuality in sponges. Pp. 39–48 in G. Larwood and B. R. Rosen, Eds., *Biology and systematics of colonial organisms*. Academic Press, New York.

Evans, C. W., J. Kerr, and A. S. G. Curtis. 1980. Graft rejection and immune memory in marine sponges. Pp. 27–34 in M. J. Manning, Ed., *Phylogeny of immunological memory*. Elsevier/North Holland Biomedical Press, Amsterdam.

Hildemann, W. H. 1962. Immunogenetic studies of poikilothermic animals. *Am. Nat.* **96:** 195–204.

Hildemann, W. H., C. H. Bigger, I. S. Johnston, and P. L. Jokiel. 1980. Characteristics of transplantation immunity in the sponge, *Callyspongia diffusa*. *Transplantation*, **30:** 362–367.

HILDEMANN, W. H., C. H. BIGGER, P. L. JOKIEL, AND I. S. JOHNSTON. 1980. Characteristics of immune memory in invertebrates. Pp. 9–15 in M. J. Manning, Ed., *Phylogeny of immunological memory*. Elsevier/North Holland Biomedical Press, Amsterdam.

HILDEMANN, W. H., I. S. JOHNSTON, AND P. L. JOKIEL. 1979. Immunocompetence in the lowest metazoan phylum: transplantation immunity in sponges. *Science* **204:** 420–422.

HILDEMANN, W. H., P. L. JOKIEL, C. H. BIGGER, AND I. S. JOHNSTON. 1980. Allogeneic polymorphism and alloimmune memory in the coral, *Montipora verrucosa*. *Transplantation*, **30:** 297–301.

HILDEMANN, W. H., R. L. RAISON, G. CHEUNG, C. J. HULL, L. AKAKA, AND J. OKAMOTO. 1977. Immunological specificity and memory in a scleractinian coral. *Nature* **270:** 219–223.

HILDEMANN, W. H., R. L. RAISON, AND C. J. HULL. 1977. Immunocompetence in corals: issues of specificity, memory and mechanisms. Pp. 9–16 in J. B. Solomon and J. D. Horton, Eds., *Developmental immunobiology*. Elsevier/North Holland Biomedical Press, Amsterdam.

HOUCK, J. E., R. W. BUDDEMIER, S. V. SMITH, AND P. L. JOKIEL. 1977. The response of coral growth rate and skeletal strontium content to light intensity and water temperature. Pp. 425–431 in D. L. Taylor, Ed., *Proc. Third Int. Symp. Coral Reefs*, Vol. 2. Rosenstiel School of Marine and Atmospheric Sciences, Coral Gables, Florida.

JACKSON, J. B. C. AND L. BUSS. 1975. Allelopathy and spatial competition among coral reef invertebrates. *Proc. Nat. Acad. Sci. U. S. A.* **72:** 5160–5163.

JOHNSTON, I. S., AND W. H. HILDEMANN. In press. Cellular defense systems in the Porifera. In N. Cohen and M. Sigel, Eds., *The ontogeny and phylogeny of the reticuloendothelial system*. Plenum Press, New York.

JOKIEL, P. L., AND S. L. COLES. 1977. Effect of temperature on the mortality and growth of Hawaiian reef corals. *Mar. Biol.* **43:** 201–208.

JOKIEL, P. L., AND E. B. GUINTHER. 1978. Effects of temperature on reproduction in the hermatypic coral *Pocillopora damicornis*. *Bull. Mar. Sci.* **28:** 786–789.

LANG, J. 1973. Interspecific aggression by scleractinian corals. 2. Why the race is not only to the swift. *Bull. Mar. Sci.* **23:** 260–279.

LITCHFIELD, J. T. 1949. A method for rapid graphic solution of time-per cent effect curves. *J. Pharmacol. Exp. Ther.* **97:** 399–408.

MANNING, M. J., AND R. J. TURNER. 1976. *Comparative immunobiology*. Halstead Press/John Wiley & Sons, New York. 184 pp.

MCCLAY, D. R. 1974. Cell aggregation: properties of cell surface factors from five species of sponge. *J. Exp. Zool.* **188:** 89–102.

MOSCONA, A. A. 1968. Cell aggregation: properties of specific cell ligands and their role in the formation of multicellular systems. *Dev. Biol.* **18:** 250–277.

PARIS, J. 1961. Greffes et sérologie chez les éponges silicieuses. *Vie Milieu Suppl.* **11:** 1–74.

RAISON, R. L., C. J. HULL, AND W. H. HILDEMANN. 1976. Allogeneic graft rejection in *Montipora verrucosa*, a reef-building coral. Pp. 3–8 in R. K. Wright and E. L. Cooper, Eds., *Phylogeny of thymus and bone marrow—bursa cells*. Elsevier/North Holland Biomedical Press, Amsterdam.

REISWIG, H. M. 1971. *In situ* pumping activities of tropical Demospongiae. *Mar. Biol.* **9:** 38–50.

SARÀ, M. AND J. VACELET. 1973. Ecologie des démosponges. Pp. 462–576 in P. P. Grassé, Ed., *Traité de zoologie, anatomie, systematique, biologie: Spongiaires*. Masson, Paris.

SHICK, J. M., AND A. N. LAMB. 1977. Asexual reproduction and genetic population structure in the colonizing sea anemone *Haliplanella luciae*. *Biol. Bull.* **153:** 604–617.

SNELL, G. D., J. DAUSSET, AND S. NATHENSON. 1976. *Histocompatibility*. Academic Press, New York. 401 pp.

TRENCH, R. K. 1979. The cell biology of plant-animal symbiosis. *Ann. Rev. Plant Physiol.* **30:** 485–531.

VAN DE VYVER, G. 1980. Second-set allograft rejection in two sponge species and the problem of alloimmune memory. Pp. 15–26 in M. J. Manning, Ed., *Phylogeny of immunological memory*. Elsevier/North Holland Biomedical Press, Amsterdam.

VAUGHAN, T. W. 1907. Recent Madreporaria of the Hawaiian Islands and Laysan. *U. S. Nat. Mus. Bull.* **59:** 1–427.

WEINS, J. A. 1977. On competition and variable environments. *Am. Scientist* **65:** 590–604.

WRIGHT, R. K., E. F. EIPERT, AND E. L. COOPER. 1978. Regulating role of temperature on the development of ectothermic vertebrate lymphocyte populations. Pp. 80–92 in M. E. Gershwin and E. L. Cooper, Eds., *Animal models of comparative and developmental aspects of immunity and disease*. Pergamon Press, New York.